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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

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SUBJECT: EFED Review of Relyea Paper Entitled "Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (*Hyla versicolor*) "

TO: Anthony Britten, Chemical Review Manager
Betty Shackleford, Product Manager
Special Review and Reregistration Division

FROM: Thomas M. Steeger, Ph.D., Senior Biologist
Environmental Risk Branch IV/EFED (7507C)

Through: Betsy Behl, Branch Chief
Environmental Risk Branch IV/EFED (7507C)

The Environmental Fate and Effects Division (EFED) has completed its review of the research article entitled *"Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles* (*Hyla versicolor*)" published in the February 2001 issue of the Proceedings of the National Academy of Science. The paper, authored by Rick Relyea and Nathan Mills (Department of Biology, University of Pittsburg) provides data demonstrating that prolonged sub-acute exposure of gray treefrog tadpoles to carbaryl at 3 to 4% of the reported LC50 (2.5 - 20.6 mg/L) killed 10 to 60% of the tadpoles. Furthermore, the paper claims that in the presence of "predatory cues" carbaryl was 2 to 4 times more lethal to tadpoles. The authors conclude that "under more realistic conditions of increased exposure times and predatory stress [simulated in their study], current application rates for carbaryl can potentially devastate gray treefrog populations" and that given the common mechanism of action, *i.e.*, acetylcholinesterase inhibition, of carbaryl with other widely used pesticides (carbamates and organophosphates), the "negative impacts may be widespread in nature."

While EFED concurs that biotic and abiotic effects do impact the toxicity of chemicals, we do not concur with the author's contention that their protocol is indicative of "more realistic ecological conditions" than EFED’s current battery of acute and chronic toxicity tests; all of these studies are conducted under rigidly controlled laboratory conditions and are not intended to be representative of all of the variables that may affect the toxicity of a compound in the field. Furthermore, the EFED environmental fate and ecological risk assessment chapter on carbaryl submitted in support of the re-registration eligibility decision does attempt to account for carbaryl's risk to amphibians and is to some extent protective to amphibians at the concentrations discussed in the Relyea and Mills paper. However, while the authors are correct that a cumulative assessment of the effects of all chemicals acting through a similar mode of action may be more realistic, the logistics of conducting such an evaluation would require additional resources than are currently available in EFED.

The EFED environmental fate and ecological risk assessment chapter on carbaryl contains both acute and chronic amphibian toxicity data (see **Attachment 1** for excerpt on amphibians from chapter). Although bullfrogs *(Rana catesbeiana*) are relatively inured (LD50 > 4,000 mg/Kg) to carbaryl on an acute oral exposure basis, leopard frog tadpoles *(Rana blairi*) exhibited a 90% reduction in swimming activity at carbaryl concentrations in the 3.5 - 7.2 mg/L range. The chapter notes that such an impairment would likely render the tadpoles [prey] vulnerable to predation provided the predators were not similarly impaired. Furthermore, the chapter notes that chronic exposure of southern leopard frogs *(Rana sphenocephala)* to carbaryl led to developmental and growth effects and that the long-term effects of short-term carbaryl exposures to amphibians during critical life stages was uncertain and could potentially lead to population-level effects. Therefore, the EFED risk assessment does discuss qualitatively the potential susceptibility of amphibians following both acute and chronic exposure to carbaryl.

EFED does not typically evaluate risk to aquatic animals on a species-by-species or class-by-class basis but rather relies on surrogate species as representatives of broad ranges of aquatic organisms. As with most screening-level risk assessments conducted by EFED, the carbaryl chapter used fish toxicity data as a surrogate for amphibians. Toxicity values for freshwater fish ranged from 0.25 to 20 mg/L; the most sensitive species, *i.e.*, Atlantic salmon (*Salmo salar*) with a 96-hour LC50 value of 0.25 mg/L, was selected for calculating risk quotient (RQ) values used in EFED's assessment of ecological risk to freshwater vertebrates. The salmon LC50 value represents roughly 10% of the lower LC50 range (2.5 to 20.6 mg/L) for amphibians reported in Relyea and Mills paper. Given that EFED's levels of concern (LOC), *i.e.*, the ratio of expected environmental concentrations (EEC) to the LC50 value, for endangered is 0.05, if the EEC was greater than 0.01 mg/L, it would exceed EFED's LOC. Therefore, the ecological risk assessment for aquatic vertebrates is protective for species with 96-hour LC50 values greater than 0.01 mg/L. (0.04% of the range reported by Relyea and Mills).

EFED concurs with the study authors that biotic and abiotic effects can impact the toxicity of pesticides and that it is difficult to account for these effects on the basis of the limited laboratory tests that are typically available for evaluating the effects of pesticides. EFED also concurs with the authors that chemicals with similar modes of action may have additive toxicities and that cummulative assessments may better account for toxicity; however, the practicality of implementing such evaluations is limited for screening-level assessments.

EFED is uncertain regarding how representative the Relyea and Mills article is of field effects though or of the direct effects of carbaryl and predatory cues. The experimental design included 10 tadpoles in 10-liter polyethylene tubs containing filtered tapwater. In a 10-day static renewal study, they changed water on days 3 and 7. In 16-day static-renewal exposures, they changed water every 4 days. Water quality parameters (dissolved oxygen, temperature, pH and ammonia) were measured midway through the 16-day exposure studies. Predator treatments consisted of a larval salamander (*Ambystoma maculatum*) housed within a 250-ml plastic cup, covered with a fiberglass window screening, in each of the exposure tanks; controls consisted of the plastic cup alone. Nominal carbaryl concentrations ranged from 0.045 to 0.54 mg/L; both negative and solvent (acetone) controls were run concurrently. The results demonstrate that increased ammonia concentrations were associated ($P < 0.0001$, range of means = 0.21 - 0.99 mg/L) with carbaryl concentration, an effect attributed to the presence of dead tadpoles and excess unconsumed food. A regression analysis of survival against ammonia was significant ($P < 0.001$, but not particularly predictive ($R^2 = 0.395$). Predators had no effect on ammonia ($P > 0.1$) and only had small effects on oxygen and pH (9% decrease in oxygen, $P < 0.0001$; 5% decrease in pH, $P = 0.019$). Given that water quality parameters were only measured midway through the study and that both tadpoles and thus feeding rates were likely increasing throughout the study, ammonia levels may have been considerably higher toward the end of the studies. Thus it is unclear whether ammonia, pH and dissolved oxygen had an effect on the toxicity of carbaryl to tadpoles. It is noteworthy that the Relyea and Mills data showed precipitous declines in tadpole survival after 5 days of exposure.

Although it is difficult to design a study that can accurately reflect field conditions and particularly predator-prey relationships, EFED is not convinced that the Relyea and Mills study could be interpreted as more representative of field conditions. Typically, prey demonstrate predator avoidance behavior in the presence of a perceived threat. In this study, tadpoles were unable to escape their perceived threat; predatory cues, *i.e.*, seeing a predator (visual cues) may have protracted their response well beyond the chemical cues released following the salamander's consumption of tadpoles. It is questionable whether tadpoles would have remained in view of a potential predator under more realistic conditions.

In refined ecological assessments, EFED oftentimes has mesocosm study data available to assess the risk of pesticides under "field conditions". These studies, while considerably more expensive that the Relyea and Mills protocol, may represent the most accurate reflection of controlled field studies. It is interesting to note though that while mesocosm studies may yield LC50 values similar to laboratory studies, they rarely provide LC50 values showing enhanced toxicity. Test species within these studies are better able to rely on compensatory mechanism to shield themselves from the toxic effects of chemicals.

In addition, the environmental fate of pesticides is often different under field conditions. Under alkaline conditions, *i.e.*, pH > 7, carbaryl undergoes hydrolysis with half-lives ranging from 0.15 to 12 days. While Relyea and Mills accurately note carbaryl's susceptibility to hydrolysis, they fail to mention that under aearobic conditions, carbaryl is also microbially degraded in the aquatic environment with a half life of approximately 5 days. It is likely that gray treefrogs in the Relyea and Mills study were exposed to carbaryl concentrations considerably lower than nominal after 3 to 4 days. Thus the actual exposure regime may have been more representative of pulsed exposures to declining concentrations of carbaryl and increasing concentrations of ammonia. While it is clear that predators had an effect on the response of tadpoles to the exposure regime, EFED does not concur that the test results are representative of the effects of predation on carbaryl toxicity alone.

EFED concurs with Relyea and Mills that both biotic and abiotic factors impact the toxicity of pesticides and that current screening methods do not account for the full range of these effects nor do screening level assessments take into account aggregate effects from exposure to chemicals with similar modes of action. Screening-level assessments attempt to identify where EFED's LOCs are exceeded and where EFED has uncertainties regarding risk. With respect to amphibians, the chapter discusses the likelihood of acute and chronic effects from current uses of carbaryl.

Attachment 1. Excerpt on Amphibians from the Environmental Fate and Ecological Risk Assessment for the Reregistration of Carbaryl Chapter

According to an available supplemental study with a 50% carbaryl formulation, the LD_{50} for the bullfrog (*Rana catesbeiana*) is greater than 4,000 mg/kg, or practically nontoxic (MRID 00160000). A single acute exposure of plains leopard frog tadpoles (*Rana blairi*) to carbaryl concentrations in the 3.5 - 7.2 mg/L range led to a 90% reduction in swimming activity, including sprint speed and sprint distance, activity ceasing completely at 7.2 mg/L (Bridges 1997). This reduction in activity and swimming performance may result in increased predation rates and, because activity is closely associated with feeding, may result in slowed growth that could lead to failure to complete metamorphosis. Acute exposure to low carbaryl levels may not only affect immediate survival of tadpoles but also impact critical life history functions.

On a chronic basis, carbaryl has been shown to have the potential to adversely affect amphibians. In a recent study, nearly 18% of southern leopard frog (*Rana sphenocephala*) tadpoles exposed to carbaryl during development exhibited some type of developmental deformity, including both visceral and limb malformations, compared to a single deformed (< 1%) control tadpole demonstrating that carbaryl exposure can result in amphibian deformities (Bridges, 2000). Although the length of the larval period was the same for all experimental groups, tadpoles exposed throughout the egg stage were smaller than their corresponding controls. Because exposure to nonpersistent chemicals may last for only a short period of time, it is important to examine the long-term effects that short-term exposure has on larval amphibians and the existence of any sensitive life stage. Any delay in metamorphosis or decrease in size at metamorphosis can impact demographic processes of the population, potentially leading to declines or local extinction.